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TRANSACTIONS

Antiscorbutic Activity of Ascorbic Acid isolated from Japanese Green Tea.

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The author has briefly stated in his previous paper⁽¹⁾ that guinea pigs fed on a basal diet deficient of vitamin C for two weeks became ill of scurvy, but survived 5 weeks more, recovering from scurvy and restoring their normal growth, when they were administered with 0.8 mg of an ascorbic acid preparation from Japanese green tea *per os* daily, as supplement to the basal diet, while negative control animals fed only on the basal diet died of severe scurvy within three weeks. This therapeutic test commenced on the 4th day of May 1934 was continued for 119 days. The general procedure used in this test was just the same as that reported in the previous paper.⁽¹⁾ The following chart shows the results so obtained: 3 animals, No. 118, 119 and 120, as negative controls, took the basal diet only but soon died, showing gradually a great drop of weight. No doubt, they all had symptoms of scurvy. The test animals, No. 115, 116 and 117, were given daily both the basal diet and 0.8 mg of the ascorbic acid preparation. Of them, No. 117 died on the 95th day. The postmortem examination, however, did not suggest any proof that this death was due to scurvy. The other two animals, weighing 500 g and 417 g on the 91th and 119th day respectively, were chloroformed and, on autopsy, were found entirely free from scurvy.

These results evidently show that ascorbic acid from Japanese green tea is also antiscorbutically effective on guinea pigs by giving them a daily dose of 0.8 mg of the acid.

At the same time, test was made on an ascorbic acid preparation isolated from Natumikan juice⁽¹⁾ (a kind of Japanese lemon, *Citrus Aurantium*, L. var. *sinensis*, Engl.) for antiscorbutic activity. In this case the test was carried out for 27 days from the commencement of administering ascorbic acid.

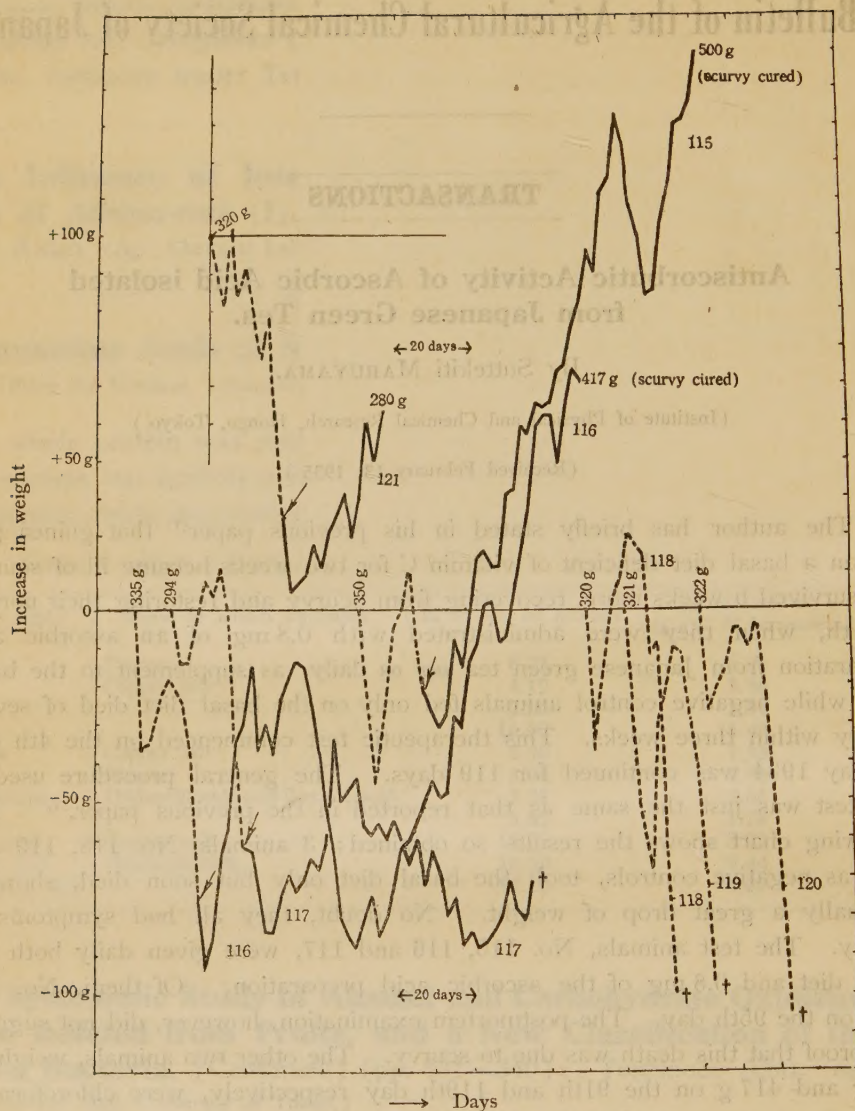


Chart 1.—Growth-curves of guinea pigs fed on a vitamin-C-deficient basal diet with and without ascorbic acid supplement. Animals No. 115—117 took an ascorbic acid preparation isolated from Japanese green tea and No. 121, that from Natumikan juice, each preparation being given in a dose of 0.8 mg daily. No. 118—120 as controls took no supplement. The lines denote: ——— basal diet alone, ——— basal diet and supplement.

Nevertheless, the fact that the animal, No. 121, fed on a daily allowance of 0.5 mg of the preparation was gaining weight and restoring liveliness during this short period, is a sufficient proof for the existence of antiscorbutic potency in the preparation, since it is similar to that already observed in other cases.

All the results shown in this paper and the previous one⁽¹⁾ allow the author to conclude the following two points:

- (1) The antiscorbutic potency of such vegetables, as Daikon juice (Daikon, *Rhapanus Sativus*, L.), Natumikan juice and Japanese green tea which the author has studied, is due to ascorbic acid contained in these vegetables.
- (2) Ascorbic acid is identical with vitamin C.

This work has been carried out under the supervision of Professor Umetaro Suzuki, to whom the author greatly owes.

LITERATURE

- (1) S. Maruyama: Sc. Pap. I. P. C. R., **24**, No. 518, 287—303 (1933); Bul. Agr. Chem. Soc. Japan, **10**, 184—200 (1934).

ABSTRACTS

from

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Studies on "Miso". VI.—Again on the changes during ripening. (pp. 349~354): By Yosito SAKURAI. (Institute of Dietary Science, Tokyo, Japan.)

On the chemical constituents of Kanzantiku (Arundinaria Hindsii, Makino). (pp. 355~356): By K. YOSHIMURA and I. YAMASHITA. (Agr. College of Kagoshima, Japan.)

(1) The seed of Kanzanchiku (Arundinaria Hindsii, Makino) resembles much unpolished rice on the general constituents, and so we can recognize it is not inferior to the latter in the nutritional value.

(2) The quantities of organic bases isolated from 3.5 kg sample are as follows:

Betain (chloraurat)	0.3 g	Cholin (chloraurat)	0.5 g
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Biochemical Studies on "Sotetsu", the Japanese Sago Plant.
III.—Enzyme-chemical Studies on a Constituent of "Sotetsu" containing Form-
 aldehyde (1). (pp. 357~364): By Kotaro NISHIDA and Aritomo YAMADA.
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**Fermentation of Arabinose and Fucose by Aspergillus ory-
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Studies on Amylosynthase. XXV.—Studies on Zymogen (1) Zymogen of Yeast-Amylosynthase. (pp. 391~394): By Toyosaku MINAKAWA. (Agr. Chemical Laboratory, Tokyo Imperial University.)

Studies on Amylosynthase. XXVI.—Studies on Zymogen (2) Zymogen of Higher plants which accumulate Starch. (pp. 395~397): By Toyosaku MINAKAWA. (Agr. Chemical Laboratory, Tokyo Imperial University.)

On Systematic Study of Alcohol and Carbohydrate Oxidizing Bacteria isolated from Fruits, and a New Classification of the Oxidizing Bacteria (Continued). (pp. 398~411): By Toshinobu ASAI. (Agr. Chem. Laboratory of Tokyo Imperial University.)

Feeding Experiments with Haemoglobin. (pp. 412~420): By Shiro MAEDA. (The Institute of Physical and Chemical Research, Tokyo, Japan.)

On the Toxic Components of Fish Liver Oils. (pp. 400~421): By Kôzô KAWAKAMI and Itsuo YAMAMOTO. (The Institute of Physical and Chemical Research, Tokyo, Japan.)

The noxious effects caused by the administration of cod liver oil or other liver oils may be attributed not to a substance but to many substances contained in these oils.

The present authors proposed to divide these toxic components into three classes, i. e. "liver oil toxin A" "B" and "C" or general fish oil toxin, cramp toxin and narcotic toxin.

The liver oil toxin A consists of those substances which are contained in the saponifiable fraction of oils and exerts nutritive disturbance or ill effects on animals, the toxicity of which disappears when hydrogenated.

The most characteristic toxin of cod liver oil belongs to this class. U. Suzuki first observed that the toxic effects of cod liver oil disappear after hydrogenation. This fact was confirmed later by S. Ueno. The present authors proved that the chief toxin of cod liver oil does not come in the non-saponifiable fraction. M. E. Bell, E. G. Gregory and J. C. Drummond

reported also on the same result.

The liver oil toxin B or cramp toxin causes severe cramps on rats when injected subcutaneously. This toxin is contained in the non-saponifiable fraction of liver oils and it is very stable against hydrogenation, oxidation and saponification. K. Takahashi was the first who drew attention to this toxin.

The toxin C or the narcotic toxin is found in the non-saponifiable fraction of cod liver oil together with the toxin B. Though its chemical properties are not yet so far studied, it can be distinguished from the latter by its physiological behavior.

The ordinary cod liver oil contains all three toxins, but certain other oils contain only one or two of them, as shown in the following table.

	Toxin A	Toxin B	Toxin C
Cod liver oil	+	+	+
Non-saponifiable fraction of cod liver oil	—	+	+
The liver oil of <i>Paralithodes camschatica</i> , a kind of crab	?	—	+
The body fat of sardin	+	—	—
Chemical properties	Saturated	Unsaturated	?

"The liver oil toxin B" or "the cramp toxin"

Though the authors could not isolate the toxin B in pure state, yet it was obtained in highly concentrated state, 1~5 mg of it caused the characteristic cramp on rats weighing 50~60 g, while 200~500 mg of the non-saponifiable fraction must be injected subcutaneously to give the same effect. When the non-saponifiable fraction of cod liver oil was repeatedly extracted with 70% ethanol or methanol, the toxin B, together with the toxin C goes to the solution, while vitamin A remains in the insoluble residue entirely free from the toxins.

When the extract is heated with fuming nitric acid at 130° for 3—5 hours, a violent reaction is caused, but the toxin B remains quite intact, while a greater part of the impurities is destroyed. The reaction mixture was now extracted with ether, and the extract was washed with dilute alkali, dried with anhydrous sodium sulphate and evaporated. The oily substance, thus obtained, was subjected to fractional distillation at 5 mm pressure. The fraction distilled at 120~140° contained the toxin B in highly concentrated state. 1~5 mg being sufficient to cause the characteristic cramps on rats weighing 50~60 g. The analysis gave: C 75.45% H 10.65%.

The toxin B is soluble in ordinary organic solvents and 90% phosphoric acid, its strong resistance to hot conc. nitric acid, resembles very much to fenchon, camphor or diketo-camphan, but they are less toxic than this toxin. 5-Oxocamphor resembles more in its toxicity but is not identical.

Vitamin A has no toxic action

The present authors have emphasized that the toxin B and vitamin A

are entirely different substances, and pointed out the difference between the solubilities and their boiling points. Further, it was shown that the toxin B can be obtained free from vitamin A and vitamin A free from any toxin.

Distribution of the toxin B

The toxin B is contained in many other liver oils, but not in vegetable oils. The distribution of the toxin is given in the following table. (The quantity of the toxin B sufficient to cause the characteristic cramps on rats weighing 50~60 g by the subcutaneous injection and death within 1~2 hours, is taken as "one B toxin unit.")

Liver oil of	Number of B toxin units contained in 100 g of oils	
	More than 33 units	
<i>Stereolepis ishinagi</i>	More than 33 units	
" <i>Balaenoptera borealis</i>	" " 10 "	
" <i>Hyppoglossus stenolepis</i>	6	
" <i>Sebatodes flammeus</i>	More than 2 units	
" <i>Gadus macrocephalus</i>	1~2	
" <i>Theragra chalcogramma</i>	0.5~1.0	
" some Japanese calamarys	Nothing	
" <i>Paralithodes camtschatica</i>	Null	
" ox	"	
Body fat of sardin	"	
Arctic sperm oil	"	
Egg Yolk oil (hen's)	"	
Oil of Japanese mushroom shiitake, <i>Cortinellus</i> <i>shiitake</i>	"	
Seed oil of <i>Gardenia grandiflora</i>	"	

On New Crystalline Derivatives of Vitamin A. (pp. 422~430):

By KOZO KAWAKAMI. (The Institute of Physical and Chemical Research, Tokyo, Japan.)

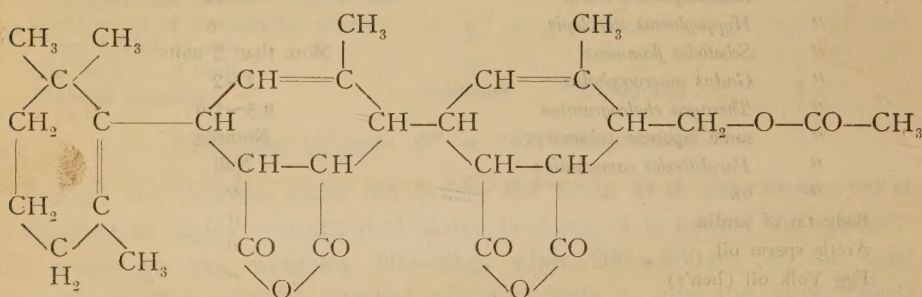
The present author has recently prepared two crystalline derivatives of vitamin A by condensing acetyl-biosterin with maleic acid anhydride. They are isomer to each other and correspond to the formula $C_{30}H_{36}O_8$. One of them forms colourless rhombic plates m.p. 261~262° hardly soluble in chloroform, and can be recrystallized from glacial acetic acid. To this the name "acetyl-sukesioic acid anhydride" was given.

The other forms colourless rectangular plates m.p. 221~222° easily soluble in chloroform, and can be recrystallized from glacial acetic acid. It is called "acetyl-ishinagiic acid anhydride". In the same manner "benzoyl-sukesioic acid anhydride" was prepared from benzoyl-biosterin, but in this case no isomer could be obtained. The formula of this compound was found to be $C_{35}H_{38}O_8$:

These three crystalline derivatives have each 8 atoms oxygen in 1 molecule, among which two atoms are presumed to be present in the form of acetyl- or benzoyl-ester and the remaining 6 atoms in the form of maleic acid anhydride rest, if two molecules of maleic acid anhydride are condensed with 1 mol acetyl- or benzoyl-vitamin A.

Assuming the P. Karrer's structural formula for vitamin A, $C_{20}H_{30}O$ to be correct, the following formula has been assigned to the crystalline derivatives mentioned above.

S. Hamano in this laboratory has recently prepared a highly purified vitamin A fraction from sukeso liver oil. The acetylated product gave also a crystalline derivative with maleic acid anhydride after the present author's method and it was proved to be identical with the acetyl-sukesoc acid anhydride mentioned above.



Acetyl-sukesoc acid anhydride m p $261\sim 262^\circ$ and its isomer $221\sim 222^\circ$.

The present author has also prepared purified hydro-vitamin A from cod liver oil and proved it to have the formula $C_{20}H_{38}O$. This gives further support to the Karrer's formula for vitamin A. i. e. $C_{20}H_{30}O$.